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RESEARCH ARTICLE

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Effects of subcutaneous progesterone injection as a short-time estrus synchronization protocol in ewes: a preliminary study

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ABSTRACT

The present study aimed to evaluate the effect of short-time progesterone injection subcutaneously (SC) on estrus synchronization of Baluchi ewes. All ewes received one dose of PGF2α (15 mg) and then were divided into two groups. In the P4 group, a combination of progesterone and propylene glycol was subcutaneously injected twice at the three-day interval, and a vaginal sponge was used in the control group for 6 days. On day 6, all ewes received 400 IU of eCG. 48h after eCG injection, two rams were introduced into the flock. Blood samples were taken daily from Day 0 to Day 13 to measure serum progesterone concentration. Ultrasonography was used to observe ovaries and monitor their changes at three-day intervals. In P4 and control groups, the estrus rate was 88.9% and 100% for P4 and control groups, respectively; the duration of estrus for the P4 and control groups was 8 and 8.5 days, respectively (p >0.05). No significant difference was observed in the size of the largest follicle and the number of follicles more than 2 mm in diameter between the treatment and control groups. Further studies with some changes and modifications are required for gaining acceptable fertility and prolificacy rates.

Keywords

Estrus, synchronization, ewe, progesterone, short-time protocol

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Abbreviations

CIDR: Controlled internal drug release
eCG: equine serum gonadotrophin

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Introduction

Several protocols are used to induce and synchronize estrus or/and ovulation in ewes. One of the most common methods used for estrus synchronization in flocks is using intra-vaginal progesterone combined with eCG treatment. In this method, intra-vaginal progesterone was used to simulate corpus luteum (CL) which its removal coincident with eCG injection can result in ovulation. Insertion of a CIDR in anestrus goats leads to an increase in serum progesterone concentration (more than 5 ng/ml) for 3 or 4 days, which is higher than that observed in the middle stage of the physiological luteal phase. After 6-day treatment, the concentration of serum progesterone has been reduced to less than 2 ng/ml and remained low until CIDR removal [1]. The profile of serum progesterone with the above-mentioned treatment protocols is different from what is observed during the normal estrous cycle; low at the beginning which increases and remains high until luteolysis (the end of the cycle). In ewe, sub-luteal level of progesterone leads to excessive growth of the largest follicle and its persistence, which causes an increase in the age of ovulatory follicles. In old methods, progesterone was used as long as corpus luteum existed (12 days), regardless of the stage of the cycle or follicular state of the ovary. Therefore, 12-day progesterone treatment could lead to ovulation of old follicles in those ruminants [2].

Long-term progesterone treatment effectively results in estrus synchronization but leads to different pregnancy rates. These protocols were designed before the 1990 decade, which is not in agreement with the current understanding of follicular dynamics [3].

Sareminejad et al (2014) reported that estrus rates in three study groups, using MAP sponge for 6 days, using MAP for 12 days, and a control group, were 93.33%, 91%, and 5.26%, respectively ($p > 0.05$) [4]. Ataman et al (2006) reported estrus rate during the breeding season; by using progesterone sponge for 7 or 12 days, the estrus rate was 100% and that of the non-breeding season was 93.3% and 86.6%, respectively ($p > 0.05$) [5]. Contreras-Solis et al (2008) indicated that subcutaneous progesterone injection with olive oil and propylene glycol could maintain plasma progesterone at a high level for 41 and 89 hours, respectively. In the propylene glycol group, plasma progesterone concentration was higher than 0.5 ng/ml for 52 hours [6]. Various investigations showed that treatment with a high dose of progesterone in reproductive programs for a short period could improve the control of follicular dynamics and pregnancy rate in small ruminants [7].

The present study aimed to investigate a low-cost and practical method for delivering progesterone as part of an estrus synchronization protocol in ewes.

Results

The The lowest serum progesterone concentration after eCG injection did not differ significantly between the two groups ($p > 0.05$) (88.9% vs 100% in P4 and control groups, respectively). Furthermore, the level of progesterone less than 0.5 ng/ml was considered as a criterion for ewes to be in estrus. The median, the first, and third quartiles of the duration of estrus were 8, 7, and 10.5 days in the treatment group, and 8.5, 7, and 11 days in the control group ($p > 0.05$). The median estrus duration was compared between the two groups and is shown in Figure 1. Time-interval between eCG injection and displaying estrus in the P4 and control groups were 5.3 and 4 days, respectively ($p > 0/05$).

By using ultrasonography at 25 days after removal of rams, 4 from 9 ewes in the P4 group and 8 from 10 ewes from the control group became pregnant (mean litter size was 1.25 and 1.37, respectively).

Results of the present study showed that two injections of progesterone along with propylene glycol (SC) at three-day intervals could maintain the progesterone level of more than 1 ng/ml in all ewes (Table 2). Also, the comparison of the mean progesterone concentration (ng/ml) between the two groups showed no significant difference within days 6 to 12 of the study ($p = 0.227$) (Figure 2).

Median, first quartile, and third quartile of the diameter of the largest follicle in the treatment group on day 6 were 6.27, 6.04, and 6.40, respectively. Median, first quartile, and third quartile of the diameter of the largest follicle in the control group were 6.06, 5.86, and 7.40, respectively. No significant difference was observed between these groups ($p > 0.05$) (Table 3).

The difference in follicle diameter and the number of follicles larger than 2 mm in diameter between the two groups was not statistically significant ($p > 0.05$) (Figure 3).

Discussion

According to new estrus synchronization methods, a 5- to 7-day P4 treatment seems to be sufficient for inducing estrus in ewes. The protocol used in the present study contained fewer injections and had less duration than the intra-vaginal method which made it more practical, cost-effective, simple, and quick. Dixon et al. (2006) reported that a high dosage of progesterone delivered by two CIDR-G devices for 12 days lead to considerable estrous response and a high fer-

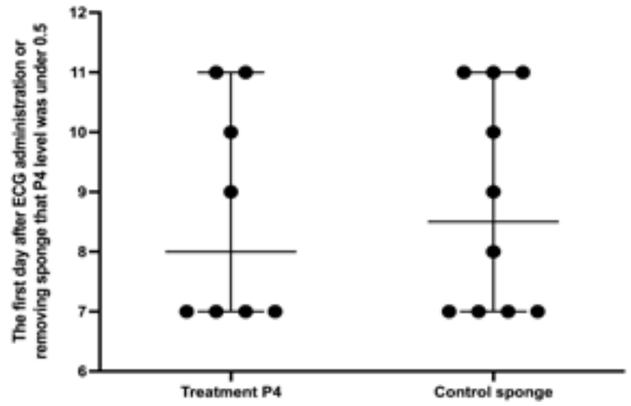

Figure 1.
Comparison of median and range of estrus time between two groups

Table 1.
Comparison of pregnancy rate, twinning and litter size between two groups

Groups	Non-pregnant sheep	single lamb	twins	Litter size
P4	5	3	1	1.25
Control	2	5	3	1.37

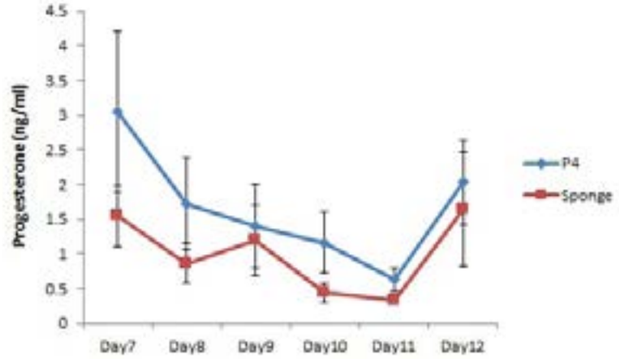

Figure 2.
Mean (±SE) of progesterone level during days 6 to 12 in control and P4 groups

Table 2.
The concentration of plasma progesterone from day 0 to 6 in P4 group (ng/ml)

Groups	Day 0*	Day 1	Day 2	Day 3**	Day 4	Day 5	Day 6
Mean	2.4	4.9	3.6	3.2	5.8	5.7	4.2
SD	2.2	2.7	1.9	1.8	1.7	1.6	2.4
Minimum	0.00	2.1	1.01	0.98	3.93	3.54	1.40
Maximum	7.026	10.44	7.44	7.49	9.74	9.06	7.92

* Day 0 was just before first progesterone injection
** Day 3 was just before second progesterone injection

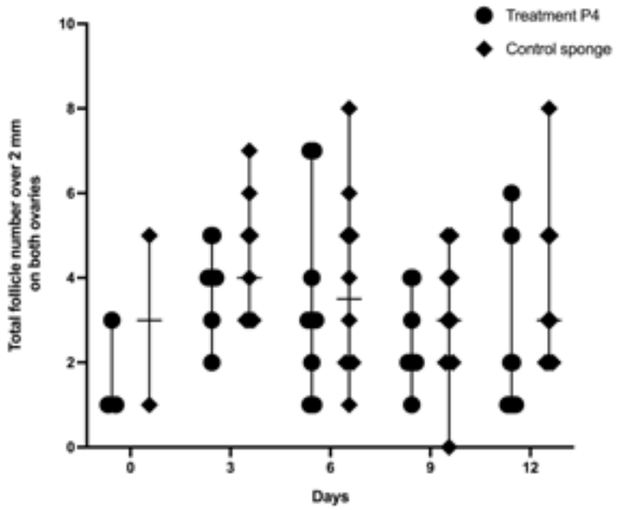

Figure 3.
The median and range of follicles over than 2 mm in two groups

Table 3.
Comparison of the largest diameter of the follicle (cm) from day 6 to 12 between two groups

Groups	Min	First quartile	Median	Third quartile	Max
P4	4.24	6.04	6.27	6.40	6.85
Control	4.62	5.86	6.06	7.40	8.81

tility rate. The mean plasma level of progesterone in ewes received two CIDRs simultaneously in their vagina for 12 days remained more than 1.4 ng/ml with a 97.7% estrus rate [8]. Pearce et al. (1985) reported that intra-vaginal sponges impregnated with 200 mg or 400, 500, or 600 mg progesterone resulted in the maintenance of plasma progesterone concentration of 1.5-4.9 ng/ml over a 12-day insertion period and create a condition similar to natural luteal phase (1.9-6.9 ng/ml) [9]. Results of the present study showed that in the P4 group the mean serum progesterone level during 6 days of synchronization protocol was 3.2-5.8 ng/ml with the minimum and maximum of 1.01 and 10.44 ng/ml. Considering mean progesterone concentration during the synchronization program, it showed that subcutaneous injection of progesterone in combination with propylene glycol could be an effective method for estrus synchronization in ewes. In the previous-mentioned study, 200 mg of progesterone was dissolved in corn oil and injected intramuscularly twice at 2.5 days intervals. Results showed that all ewes gave birth during the first 8 days of the parturition period, which indicated proper estrus synchronization [10]. In the present study, the estrus rate in the treatment and control groups was 88.9% and 100%, respectively; which no significant difference was observed between the two groups. Also, the median of the duration in which progesterone level remained less than 0.5 ng/ml in P4 and control groups were 2 and 2.5, respectively; moreover, the time that progesterone level reached its lowest level was day 3.5 and day 4.5 in P4 and control group, respectively. Robinson et al. removed sponge consisting of 10, 20, and 40 mg progesterone after 12 days and reported 29, 43-48, and 48-53 hours for the estrus occurrence peak, respectively which showed a relative delay in the peak of estrus occurrence, representing that by increasing progesterone, a rise in the plasma progesterone level was observed [11].

In this study, no difference in the size of the largest follicle and the number of follicles larger than 2 mm was observed during the observation of ovaries and follicles. This relative similarity in terms of the diameter of the largest follicles in the two groups was proportionate and was following the relative similarity in the estrus rate between the two groups. Johnson et al. (1996) indicated the larger follicle diameter in low progesterone concentration (1 ng/ml>) compared

to high progesterone concentration (1 ng/ml<) [12].

Results of the present study showed that plasma progesterone concentration in the P4 group was more than 1 ng/ml during the estrus synchronization protocol while we could not measure the plasma progesterone concentration during the synchronization protocol in the sponge group. An investigation showed that sheep with a low level of plasma progesterone (less than 1 ng/ml) had larger and older follicles compared to sheep with a high level of plasma progesterone, which resulted in a lower pregnancy rate [12]. Furthermore, the progesterone concentration during estrus in the control group was lower than in the P4 group which may lead to a higher estrus rate and pregnancy rate in the control group. In the artificial insemination technique, if the estrus occurs on certain days, the high fertilization rate will be predictable while in natural breeding programs shorter duration of estrus in the P4 group may lead to a lower pregnancy rate.

In conclusion, two subcutaneous injections of progesterone as a synchronization protocol may be used as a practical cost-effective method of estrus synchronization however, further studies with larger sample sizes and minor changes are required to achieve an acceptable fertility and prolificacy rates.

Materials & Methods

The present study was performed in the farm animal and poultry research center of the Ferdowsi University of Mashhad, Mashhad, Iran (latitude 35° 43' to 37° 8'N and the longitude 59° 4' to 60° 36'E). The selected ewes were not lactating and the study was performed at least three months post-lambing, also they had no observable general or reproductive disease. 5 ewes were excluded from the study due to illness or other problems and the investigation started with 19 sheep. The diet of ewes was balanced based on NRC. Ewes were divided into two groups randomly. The treatment group (P4): consisted of 9 ewes, which received 15 mg prostaglandin intramuscularly (vetalyse, Aburaihan, Iran) and after 3 hours 25 mg progesterone (Vetagestrone, Aburaihan, Iran) with 4mL propylene glycol injected subcutaneously. After 3 days, 400 IU eCG was injected intramuscularly and after 48 h, two rams were introduced into the flock and stayed for 5 days.
The control group: consisted of 10 ewes that received one dose of 15 mg prostaglandin (vetalyse, Aburaihan, Iran) intra-muscularly and after 3 hours an intra-vaginal progesterone sponge consisting of 60 mg MPA (Sponjavet, Hipra, Spain) was used for 6 days and 400 IU of eCG was injected intramuscularly after sponge removal (Gonaser, Hipra, Spain). After 48 h, two rams were introduced into the flock for 5 days.

MINDRAY DP-6600VET and rectal linear probe with frequencies of 7.5 and 10 MHz were used for ultrasonographic evaluation of ovaries. All ewes were 15-20 hours off-feed before the ultrasonographic examination. Ultrasonography of both ovaries was done from day 0 until day 13 and the follicular map was recorded. The follicles larger than 2 mm in diameter were mapped. Blood samples were collected daily for 13 consecutive days from the first day. The blood samples were centrifuged immediately at 3000 × g for 20 min, and collected blood serums were stored at −20 °C until progesterone measurement. The concentration of progesterone was determined by ELISA. (DRG, Germany). The P4 concentration of less than 0.5 ng/ml was considered as a criterion for ewes to be in estrus.

A Chi-square test was used to compare the estrus rate between the two groups. Mean progesterone levels were compared between two groups by using the ANOVA test. Mann-Whitney U test was used to compare the average diameter of the largest follicles, the number of follicles larger than 2 mm, and to compare the estrus duration and the number of embryos and lambs between two groups. Statistical analysis was performed using SPSS software. A p-value less than 0.05 was considered statistically significant.

Authors' Contributions

Research concept and design: BK, MR; Analyzed the data: MA; Performed the experiments: BV, BK, MR; wrote the paper: BV, BK, MR; All the authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no conflict of interest.

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